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Evaluation of fungal growth on cellulose-containing and inorganic ceiling tile

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Abstract

Buildings with poor indoor air quality **(IAQ)** frequently have many areas with surface fungal contamination. Studies have demonstrated that certain fungal genera (e.g., *Cladosporium*, *Penicillium*, and *Stachybotrys*) are able to grow on building materials **such** as wallpaper, drywall, and ceiling tiles, particularly after water damage has occurred. Due to the increasing **awareness** of sick building syndrome (SBS), it has become essential to identify building materials **that** prevent the interior growth of **fungi.** The objective of this study was to identify building materials that would not support the growth of certain fungal genera, regardless of whether an external food source was made available. The growth of three fungal genera (*Cladosporium*, *Penicillium*, and *Stachybotrys*) was evaluated on cellulose-containing ceiling tile (CCT) and inorganic ceiling tile (ICT). Both types of ceiling tile **were** exposed to environmental conditions which can occur inside a building. Our results show that ICT did **not** support the growth of these three fungal genera while CCT did. Our data demonstrate that **ICT** could serve as an ideal replacement for CCT.

Key words: ceiling tile, Conidia, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, Sick building syndrome, *Stachybotrys chartarum*

Introduction

Sick building syndrome (SBS) **is** a term commonly used to describe the consequences of poor indoor **air** quality **(IAQ).** This term was first coined in 1982 to describe the multitude of symptoms commonly observed in this phenomenon [1]. These include difficulty in breathing, allergic rhinitis, watery eyes, headaches, and flu-like **symptoms** [2].

The cause(s) of SBS have been difficult to elucidate and most workers in this field feel that multiple factors could be involved [3]. Early researchers thought that the most **likely** causes of SBS were exposure to higher than normal levels of certain known compounds **(sulfur** dioxide, nitrogen, hydrocarbons and chemicals released by **new** buildings and/or **their** materials), or known or suspected carcinogens such as environmental tobacco smoke, asbestos, radon and formaldehyde [4, 5].

However, evidence in recent years is mounting that fungi and their products are associated with S3S and **poor** IAQ [6–14]. Fungal growth **in** indoor environments has been demonstrated to produce allergic:, in the building's occupants [8, 11–14]. We have recently shown that there is a correlation between the prevalence of certain fungi (*Penicillium* and *Stachybotrys* species) and SBS in public schools IS]. We also demonstrated that even though fungal profiles in the outdoor air are constantly changing, indoor air fungal profiles in "sick" buildings tend to remain unchanged [15]. In addition, we have **demonstrated** thut intranasal instillation of viable *Penicillium chrysogenum* conidia will induce asthma-like allergic responses in an animal model [16].

Fungal growth on building materials is thnught to play an important role in **SBS** [17]. Ceiling tile and drywall are common sources **of** fungal growth in SBS.

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surface of each tile. The control tiles received 1 ml of sterile PBS. The jars containing the tiles were put into a disinfected chamber with HEPA filtered air flowing through it. The tiles were evaluated over a course of time to determine when fungal growth developed on the CT placed in standing water and those that only experienced one wetting event.

Statistics

A one-way analysis of variance (ANOVA) was used to test the significance of difference in growth of the various fungi on CCT and ICT. A Mann-Whitney Rank Sum Test was employed to rest the significance between the CCT and ICT. A P value of less than 0.05 was the minimum level of significance [19]. Fungal concentrations were reported as the mean ± standard deviation (SD).

Kesults and discussion

Fungal conidin from *C. cladosporioides*, *S. chartarum*, and *P. chrysogenum* were incubated at 55% RH and 25 °C fora period of 7 to 10 days to evaluate the potential of building materials to support fungal proliferation. The viable conidial load that each CT was inoculated with was 5.80 x 10^4 CFU for *C. cladosporioides*, 3.90 x 10^6 CFU for *P. chrysogenum*, and 1.40 x 10^4 CFU fnr S. *chartarum*. As can be seen in Figure 1, after 7 days of growth on the CCT, *C. cladosporioides* had significantly multiplied ($P < 0.05$)to 2.86 x 10^6 CFU (standard deviation, 8.69 x 10^5), while the same organism significantly decreased ($P < 0.05$) in number on ICT to 2.17 x 10^2 CFU (SD. 2.58 × 10^2) in this same period of time. *P. chrysogenum* significantly increased ($P < 0.05$) from 3.90 × 10^6 CFU to 2.01 x 10^7 CFU (SD, 4.99 × 10^6) on CCT but there were no detectable *P. chrysogenum* CFU after 7 days of growth at 80% RH and 25 °C on ICT. Finally. *S. chartarum* significantly increased ($P < 0.05$) from 1.40 x 10^4 CFU on CCT to 4.35 x 10^6 CFU (SD. 5.64 x 10^6) after 7 days at 80% RH and 25 °C, while this same number of *S. chartarum* conidia when placed on ICT significantly decreased ($P < 0.05$) to 4.41 x 10^2 CFU (SD. 4.02 x 10^2) after 7 days. These results show that ICT inhibits the growth of fungi thought to he important in SBS and poor IAQ.

The next step was to determine if ICT. contaminated with an external food source. would support fungal proliferation. Figure 2 shows the results of this

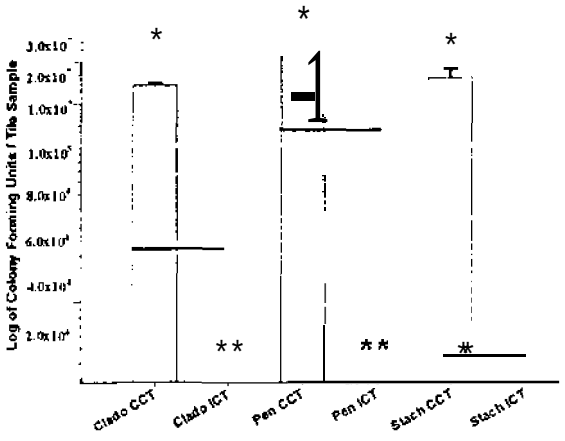

Figure 1. Colony forming units of three different fungal genera on cellulose-containing ceiling tile (CCT) and inorganic ceiling tile (ICT) exposed to fungal conidia and incubated for 7 days. Horizontal bars represent original conidia concentrations. Error bars represent standard deviation. The single asterisk (*) indicates a significant increase in the number of viable spores harvested from the tiles compared to the original inocula, and the double asterisk (**) indicates a significant decrease in the number of viable spores harvested from the tiles compared to the original inocula. A Mann-Whitney rank sum test was utilized ($P < 0.05$) to compare the inocula to the conidia harvested from the tiles. Clado signifies *Cladosporium cladosporioides*, Pen signifies *Penicillium chrysogenum*, and Stach signifies *Stachybotrys chartarum*.

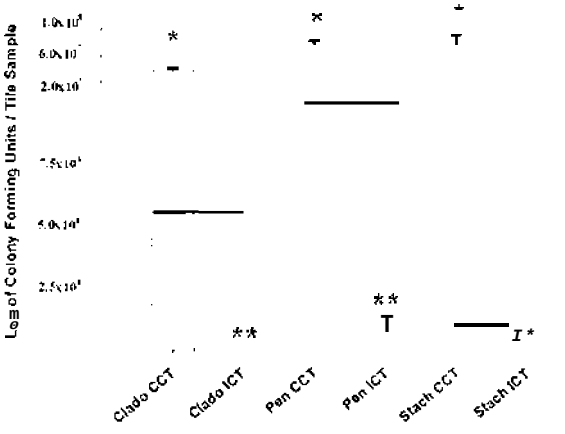

Figure 2. Colony forming units of three different fungal genera on CCT and ICT with 400 µl of TSB exposed to fungal conidia and incubated for 7 days. Horizontal bars represent original conidia concentrations. Error bars represent standard deviation. The single asterisk (*) indicates a significant increase in the number of viable spores harvested from the tiles compared to the original inocula, and the double asterisk (**) indicates a significant decrease in the number of viable spores harvested from the tiles compared to the original inocula. A Mann-Whitney rank sum test was utilized ($P < 0.05$) to compare the inocula to the viable conidia harvested from the ceiling tiles. Clado signifies *Cladosporium cladosporioides*, Pen signifies *Penicillium chrysogenum*, and Stach signifies *Stachybotrys chartarum*.

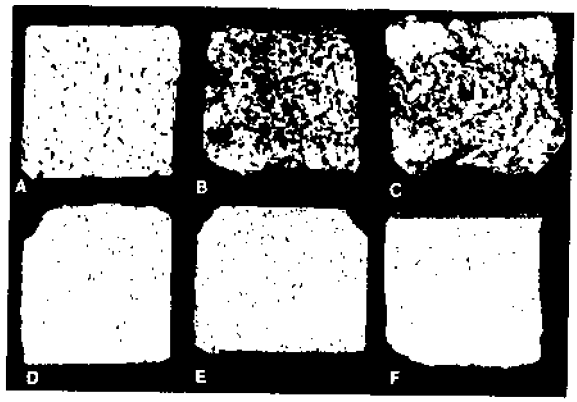

Figure 3. Fungal growth on cellulose-containing ceiling tile (CCT) and inorganic ceiling tile (ICT). CCT (B, C) and ICT (E, F) were inoculated with 3.0×10^4 CFU of *S. chartarum* and incubated for 7 days at 25 °C and 80% RH. 3A and 3D represent uninoculated CCT and ICT, respectively. 3B and 3E represent CCT and ICT inoculated with 3.0×10^4 CFU of *S. chartarum* plus 0 μl of TSB, respectively. 3C and 3F represent CCT and ICT inoculated with 3.0×10^4 CFU of *S. chartarum* plus 400 μl of TSB, respectively. These results are representative of all of the fungi used in this study.

study. When 5.80×10^4 CFU of *C. cladosporioides* conidia were placed on CCT containing 400 μl of TSB and incubated for 7 days at 25 °C and 80% RH, the viable conidial density significantly increased ($P < 0.05$) to 2.37×10^6 CFU (SD, 1.33×10^6). Under the same conditions, the CFU of the initial inoculum significantly decreased ($P < 0.05$) to 4.35×10^2 (SD, 5.71×10^2) after 7 days on ICT containing 400 μl of TSB. When 3.90×10^6 CFU of *P. chrysogenum* conidia were placed on CCT plus 400 μl of TSB and incubated at 25 °C and 80% RH, the viable conidia count significantly climbed ($P < 0.05$) to 2.43×10^7 CFU (SD, 1.42×10^7) in 7 days. When this same inoculum of *P. chrysogenum* was placed on ICT plus 400 μl of TSB, the viable conidial density significantly decreased ($P < 0.05$) to 1.22×10^4 CFU (SD, 3.96×10^3) after 7 days incubation at 25 °C and 80% RH. Finally, when 1.40×10^4 CFU of *S. chartarum* conidia were placed on CCT plus 400 μl of TSB and incubated at 25 °C and 80% RH, the population density significantly climbed ($P < 0.05$) to 3.02×10^7 CFU (SD, 6.82×10^7) after 7 days. When this same number of *S. chartarum* conidia were placed on ICT plus 400 μl TSB, the initial inoculum significantly decreased ($P < 0.05$) to 7.35×10^2 CFU (SD, 9.16×10^2) after 7 days incubation at 25 °C and 80% RH.

These data show that it is possible to develop building materials that do not support the growth of fungal genera that are reported to be involved in SBS. The ICT used in these studies did not support the growth of *C. cladosporioides*, *P. chrysogenum* and *S. chartarum* at conditions that allowed for the luxuriant growth of these organisms on CCT (Figure 1) This striking difference in fungal proliferation on CCT and ICT can be seen in Figure 3. This figure shows *S. chartarum* proliferation on CCT in the absence of TSB (3B) and the presence of an external food source (400 μl of TSB) (3C). Figure 3 also shows the absence of fungal proliferation on ICT in the absence of TSB (3E) and the presence of 400 μl of TSB (3F). Figure 3A (CCT) and 3D (ICT) represent uninoculated ceiling tiles. These tiles were incubated for 7 days at 25 °C and 80% RH. Surprisingly, even when the ICT was "contaminated" with an additional nutrient source (TSB) that should allow for the growth of fungi, these three fungal species did not proliferate under the conditions employed in this study (Figure 2). These results seem to indicate that the ICT contains components that prevent fungal growth. However, the chemical components that prevent fungal growth are proprietary material.

Observations were made regarding the CT that were placed in standing water and those that experienced one wetting event. Qualitative analysis of these tiles indicated that *Stachybotrys* growth was apparent by the 3rd day of incubation on the surface of CCT of both the tile sitting in standing water and the tile that had experienced one wetting event. On the 5th day of incubation there was confluent Stachybotrys growth over the surface of the CCT in standing water and there was confluent *Stachybotrys* growth over the surface of the CCT by the 7th day of incubation. There was no visible growth on the surface of the ICT in standing water or those that experienced one wetting event over the seven-day incubation period. The results of these experiments seem to indicate that regardless of whether the CCT is exposed to constant wetting or only one wetting event, fungal growth is able to develop on the surface of these tiles within a seven-day incubation period.

It is of extreme importance to develop and employ building materials that do not support the growth of certain fungi. Many published studies support the concept that fungi are an important component of SBS and poor IAQ [6, 7, 9, 14–17]. We have demonstrated the role of certain fungi (most notably *Penicillium* and *Stachybotrys* species) in colonization of structures and their relationship to SBS [9, 15]. Ahearn et al. [6] reported that the air-handling units and fiberglass duct liner of the heating, ventilation, and air-conditioning systems may be heavily infested with *Penicillium* and

Cladosporium species, even in buildings without reported water intrusion. We have recently demonstrated that the inhalation of viable *Penicillium chrysogenum* conidia can induce allergic symptoms in a mouse model [16].

In our study examining public schools, we observed that water intrusion onto building materials such as drywall and ceiling tiles was one of the primary factors that led to Fungal colonization [9]. Therefore, it appears important to develop building materials that do not support the growth of fungal genera like *Cladosporium*, *Penicillium*, and *Stachybotrys*. The inhibition of Stachybotrys species growth appears to be an important component in preventing SBS and poor IAQ in the future [20]. These organisms are important due to their production of both simple and macrocyclic trichothecenes. These compounds are known to be extremely toxic [21] and their role in SBS is beginning to be closely scrutinized.

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